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TITLE: Advancing Prostate Cancer Research by Providing Summer Research Opportunities for HBCU Students at the Cancer Center at UTHSCSA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT The DOD-funded summer research experience has provided a unique opportunity for students from a Historically Black University to work at a NCI-designated Cancer Center for 10 weeks. Their research is conducted in funded prostate cancer researchers' laboratories. Before starting their research in the laboratory, the students are provided training in both laboratory methods as well as the biology of prostate cancer. During the summer, they are exposed to enrichment programs ranging from health disparity presentations to survivorship research. In addition, they obtain career guidance from the Associate Dean of the Graduate School who emphasizes the importance of this summer for exploring career opportunities. It is evident that the students contribute to the research in their laboratories by the high quality of their presentations at the end of summer poster session. Many of the students have previously considered a career in medicine, but for most of the students, this is their first exposure to a research-intensive environment. Some of the students are re-evaluating their long term career goals and exploring the possibility of either becoming a full time researcher or pursing a physician scientist degree. Thus, this summer experience is contributing to development of the next generation of prostate cancer researchers.

15. SUBJECT TERMS
Prostate Cancer Research, Historically Black University

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1. INTRODUCTION:

This program provides students from a Historically Black College and University (HBCU), Huston-Tillotson University (HTU) in Austin, Texas, an opportunity to conduct prostate cancer research at a National Cancer Institute (NCI)-Designated Cancer Center, UT Health San Antonio Cancer Center (UTHSA-CC ; formerly known as the Cancer Therapy and Research Center at the University of Texas Health Science Center San Antonio). This is a distinct partnership between these two institutions since there are only nine HBCUs in the State of Texas and only four NCI-designated cancer centers. The students participate in a 10-week program and work directly with principal investigators who are funded to conduct prostate cancer research. The program begins with some didactic and laboratory training before the students are placed in a research laboratory. During the final week of the program, the students have an opportunity to present their research at a poster session where they present their findings to faculty and students.

2. KEYWORDS:

- Prostate Cancer
- Historically Black College and University
- NCI-Designated Cancer Center

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The overall goal of this program is to develop opportunities to train minority students in southern Texas in prostate cancer research. This program provides undergraduate students with an opportunity to work at an NCI-designated cancer center and participate in state-of-the-art prostate cancer research in many focus areas including biomarkers, genetics, tumor biology, therapy and imaging. The prostate research mentors offer students research projects that encompass both basic biomedical research and translational research.

One goal of this program is to help encourage these students to learn about the graduate school experience and to equip them with research skills that will allow them to undertake careers in scientific research that will be focused on the field of prostate cancer. The program will last for 10 weeks; the first two weeks (reduced to one week in the second year of the program based on student feedback) includes course work (both didactic and laboratory based) that introduces the students to prostate cancer biology, as well as basic molecular techniques.

What was accomplished under these goals?

We were notified by the DOD that that we would be funded in spring 2016 and thus, we worked rapidly to identify students that could participate in the summer of 2016. The award actually was received in mid-June at which time we had already recruited students, confirmed mentors and selected the first year class of students. They began their research experience in early June and completed in mid-August. Below is a summary of the timeline and what has been accomplished under the award to date:

Timeline:

Task	2016				2017			
Recruit students				Completed				Completed
Confirm mentors								
Select Students								
Internship program								In Progress
Tracking &								Pending

evaluation								
Sponsor student to present at a national meeting				In Progress				**I**n Progress

The students participating in the first year of the program provided feedback in their final week at the cancer center on how their experience might have been improved. The one major concern was the limited amount of laboratory time to complete their project and so it was decided that for the subsequent year, we would reduce their didactic and laboratory training to a single week, allowing them more time in their prospective laboratories.

What opportunities for training and professional development has the project provided?

In addition to the two weeks of training described above, the students have weekly enrichment programs. These are conducted every Friday at noon and lunch is provided. The speakers for this program range from experts in Health Disparities to Cancer Survivorship. We also have a physician scientist discuss the dual M.D./Ph.D. degree with its advantages and disadvantages. The Associate Dean for the Graduate School, Dr. Nicquet Blake, also provides some career development information. Dr. Blake is one of the first presentations, so the students have an opportunity to meet with her one-on-one if they are so inclined. The students receive extensive laboratory training during the remaining weeks of the program. The new schedule for the one week of didactic training is provided below.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

We are well underway for the second year of the program. The students will be finishing up this week with the second year of summer training (completed in August 2017), and we will be working with our alumni to identify opportunities for them to present their research in other venues. It will also be important for the students as they apply for graduate and medical school that they receive letters of recommendation from their mentors in the program.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Participation in a 10-week research program is not likely to contribute significantly to new discoveries in prostate cancer; however, the goal of the program is to contribute to the development of the next generation of prostate cancer researchers. Many of the students have previously considered a career in medicine, but for most of the students, this is their first exposure to a research-intensive environment. After their summer experience, some of the students are re-evaluating their long-term career goals and exploring the possibility of either becoming a full time researcher or pursing a physician scientist degree. Thus, this summer experience is contributing to the development of the next generation of prostate cancer researchers, which will have a profound long term impact on the field.

What was the impact on other disciplines?

Training future scientists will have a broad impact on numerous disciplines, since discoveries in one field can often translate into another field.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Through this program, bright young minority students are encouraged to pursue a career in research, and this has many positive implications for society. Many students from Huston-Tillotson University had never considered a career in research because they were unaware of the numerous research opportunities, both in academics and industry. Thus, this experience may change their career trajectory, and as they interact with their family and peers, it may increase the number of future minority scientists. This is particularly important in prostate cancer where health disparities are so prevalent.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

During the first summer program, we followed the proposed schedule for two weeks of classroom training followed by 8 weeks in the laboratory. However, the students give a poster presentation during that last week, so the actual laboratory time is close to 7 weeks. During the final days of the program, the students had an opportunity to provide feedback on their summer experience. They felt it would have been more productive for them to reduce the original 2 weeks of training to a single week, thus providing more time for their actual bench work.

Shown below is the program provided in the first summer and how it was reduced for the second summer:

***Summer 2016*: Introductory Course for Summer Students enrolled in the HBCU DOD Prostate Program**

	Tuesday 5/31	***Wednesday 6/1***	***Thursday 6/2***	***Friday 6/3***	***Monday 6/6***
Week 1 *9-11 a.m. Lecture*	*Orientation Room MED 238A*	*Overview of Prostate Cancer & Prostate Biology Room MED 238A*	*Nucleic Acids Concentration and Integrity Room MED 5.573C*	*Methods Overview – Nucleic Acids Room MED 238A*	*Critical Thinking Journal Article Room 238A*
12-1 p.m.		*Lunch*	*Lunch*	*Enrichment Program*	*Lunch*
1-4 p.m. Lab (on most days)		*DNA & RNA isolation Room MED 5.573C*	***2 pm** Prostate Cancer – Clinical Perspective* *Room 238A*	*Reverse Transcription and QPCR Room MED 5.573C*	*SNP Genotyping Room 5.573C*
	Tuesday 6/7	***Wednesday 6/8***	***Thursday 6/9***	***Friday 6/10***	***Monday 6/13***
Week 2 *9-11 a.m. Lecture*	*Methods Overview – Proteins (DOK) Room STRF-2.264.00*	*Protein Isolation Room STRF 270.1 9 a.m.-12 p.m.*	*Human Genetics & Precision Medicine Room STRF-2.210.00*	*Critical Thinking Journal Article Room STRF-2.264.00*	*Critical Thinking Journal Article Room STRF-2.264.00*

				11-12pm Immunohistochemistry prep Room STRF 270.1	
12-1 p.m.	*Lunch*	*Lunch*	*Lunch*	*Enrichment Program*	*Lunch*
1-4 p.m. Lab	*Tissue Culture Techniques Room STRF 270.1*	*SDS-PAGE and Transfer Room STRF 270.1 (1-5p.m.)*	*Western Blotting Room STRF 270.1 (1-5pm)*	*Immunohistochemistry Room STRF 270.1 (1-5pm)*	*Coverslip and examine tumor slides STRF 270.1*

Summer 2017: ***Introductory Course for Summer Students enrolled in the HBCU DOD Prostate Program***

Week 1	***Monday, 6/5***	***Tuesday, 6/6***	***Wednesday, 6/7***	***Thursday, 6/8***	***Friday, 6/9***
9-11 am	*Orientation*	*Tissue Culture Overview; DNA & RNA Isolation Room MED 5.573C*	*Reverse Transcription and QPCR Room MED 5.573C*	*Protein Isolation Room MED 5.573C*	*Western Blotting Room MED 5.573C*
12-1 pm		*Lunch on your own*			*Enrichment Presentation*
1-4 pm	*Online Training*	*Medical and Bioinformatics Room MED 238A*	*Precision Medicine and Human Genetics MED 552C*	*SDS-PAGE and Transfer Room MED 5.573C*	*2-3 pm Clinical Perspective for Prostate Cancer Room MED 238A* *3-5 pm Western Blotting continued Room 5.573C*

Actual or anticipated problems or delays and actions or plans to resolve them

There was a delay in sponsoring two students to attend a national meeting. We did have one student, Anna Barbara O'James attend the Annual BKX Conference (Louisiana, March 15-18, 2017) and we anticipate supporting 3 more in the upcoming year. We are encouraging the students to attend the American Association for Cancer Research (AACR) meeting in Chicago in April 2018. The abstracts for this meeting are due December 1st. If they are unable to attend that meeting because of schedule conflicts, additional meeting opportunities will be identified and the students will receive up to $1500 from the grant toward their travel cost. Huston Tillotson University has additional funds that they plan to use if the student's expenses exceed the allotted amount.

Changes that had a significant impact on expenditures

Part of the travel funds for year one will be utilized in year 2.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Nothing to report.

- **Journal publications.**

  Nothing to report.

- **Books or other non-periodical, one-time publications.**

  Nothing to report.

- **Other publications, conference papers, and presentations.**

Trainee (bold) and co-authors	Title of Presentation	Venue at UT Health San Antonio	Additional presentations
Johnson Bobmanuel, Xiang Gu, Hakim Bouamar, Lu-Zhe Sun	Immunohistochemistry (IHC) Assay on Mouse Mammary Tissue	2016 Summer Research Poster Presentation	
Michael Esuruoso, Kyle S. Johnson, Wasim H. Chowdhury, Denise S. O'Keefe, Dean J. Bacich	The effect of metformin and folate on prostate cancer growth	2016 Summer Research Poster Presentation	
Anna Edem-Etuk, Suleman S. Hussain, Addanki P. Kumar	RPS6KB1 inhibition sensitizes prostate cancer cells to chemotherapy	2016 Summer Research Poster Presentation	Huston-Tillotson University Preview Day
A.B. O'James, A. Sidana, J. Goyal, D. Oh, G.I. Todd, M. Rahman, R. Rodriguez, W.H. Chowdhury	Common Supplements can augment the efficacy of Valproic Acid in treating Prostate Cancer	2016 Summer Research Poster Presentation	Huston-Tillotson Research Day, Annual BKX Conference (Louisiana)
Darrion Jemerson, Alexandra Sharp, Suphada Lertphinyowong, Sarah Bulin, Ph.D., and David A. Morilak, Ph.D.	Cognitive Impairment Associated with Androgen Deprivation Therapy	2017 Summer Research Poster Presentation	Huston-Tillotson STEM meeting in September 2017
Bomaonye Sokari, Saketh Amasa, Ratnesh Srivastava and A. Pratap Kumar	AMPK activates androgen receptor (AR)-signaling	2017 Summer Research Poster Presentation	Huston-Tillotson STEM meeting in September 2017
Nangah Tabukum, Yanming Wu, Kexin Xu, PhD.	Plasmid Construction- A way to study the role of ATAD2	2017 Summer Research Poster Presentation	

Tiarra Walker, Keith Ashcraft, Desiree Wilson and Denise O'Keefe	Regulation of LIN7A by Methylation in Prostate Cancer Cell Lines	2017 Summer Research Poster Presentation	Huston-Tillotson STEM meeting in September 2017

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

  Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	**Project Role**	**Researcher Identifier**	**Nearest person month worked**	**Contribution to Project**	**Funding Support**
Robin J. Leach, Ph.D.	PI	Professor, UT Health San Antonio	1	Oversaw the summer program. Involved in recruitment, selection and training of the students. Prepares progress report. Oversees accounting	National Cancer Institute and Owens Foundation
Amanda Masino, Ph.D.	Co-Investigator	Associate Professor, Huston-Tillotson University	.648	Faculty mentor for students at home institution. Helps with the recruitment and selection of students and provides mentoring throughout the year.	National Science Foundation, DOD, Office of Naval Research
Denise O'Keefe,	Co-	Associate	0.5	Mentor students	DOD, NCI

Ph.D.	Investigator	Professor		and assist with didactic training	
Teresa Johnson-Pais, Ph.D.	Co-Investigator	Associate Professor	1	Oversee the Didactic training of students during first week(s) of program. Provides some mentoring	NCI
Eva Garcia-Rodriguez	Coordinator	Research Coordinator - Senior	1.8	Administrator for whole program, communicate with student, disseminate recruitment material, facilitate student selection, managing weekly enrichments, prepared paperwork for employment and oversaw poster session	DOD, NCI
Students	See information provide in Table in Section	Undergraduates at Huston Tillotson University	2.0	Participated in summer research	Current project - DOD

Participating Students

Trainee	***Research Mentor***	***Project Description***
Johnson Bobmanuel	*LuZhe Sun, Ph.D.*	*Role of mammary stem/progenitor cell in tumorigenesis: Student will assist with immunohistochemical staining, solution making, and other routine lab chores, and help with data analysis and management, and literature review.* *Follow-Up: Preparing for the GRE and start submitting applications to graduate school before the end of this year. Proposing to apply to UT Health San Antonio along with other institutions.*
Michael Esuruoso	*Dean Bacich, Ph.D.*	*The effect of metformin and folate on prostate cancer growth and gene regulation: Student will perform tissue culture of prostate cancer cell lines, and measure growth responses of these cells in response to altered folate and metformin. Students will learn and perform real time PCR for various folate regulating enzymes. They will isolate RNA from these cells, perform reverse*

		transcriptions and determine if these the transcription of these enzymes are altered in the above treatment of the cells. Students will isolate protein from cells and perform Western Analyses. Students will measure folate content of their treated cells via a bacterial folate assay. *Follow-Up: Preparing to apply for medical school.*
Anna Edem-Etuk	*A. Pratap Kumar, Ph.D.*	*Targeted approaches for prostate cancer management: Student will assist with literature review, identifying molecules associated with radiation sensitization/resistance.* *Follow-Up: Submitted applications to various graduate schools (Medical Sciences and/or Public Health)*
A.B. O'James, A	*Ron Rodriguez, M.D., Ph.D.*	*The student will assist with cell culture and help carry out a set of experiments that will be looking at the effect of folate and common supplements people take on cell growth. In particular, the experiment will entail growing human prostate cancer cells in media containing different levels of folate, supplemented with different amounts of omega 3 fatty acid (DHA), and checking on cell growth/death by using a cell viability assay.* *Follow-Up: Preparing for the MCAT, currently a senior at Huston Tillotson University*
Darrion Jemerson	*David Morilak, Ph.D.*	*Darrion Jemerson worked on a pre-clinical project using rats to study the mechanisms and a potential treatment for cognitive impairment induced by androgen deprivation therapy for prostate cancer. He worked with a graduate student and a Research Assistant in the lab, comparing castrated male rats as a model of androgen deprivation therapy and intact controls. Within these groups, half of each were treated with a novel antidepressant drug, vortioxetine, which has beneficial effects on cognitive impairment in depression. The drug was given in the diet, and controls received standard diet. They tested the performance of the rats on the Novel Object Location test, a measure of visuospatial cognitive function, one of the more consistent deficits seen in prostate cancer patients treated with ADT, which is mediated in the hippocampus. And they measured change in the electrical response evoked in the medial prefrontal cortex by stimulating in the hippocampus, a measure of the integrity of this important pathway that is*

		involved in higher order cognitive processes. *Update: Current Junior at Huston-Tillotson University*
Bomaonye Sokari	*A. Pratap Kumar, Ph.D.*	*Understand the role of AMPKα1 in combating different stresses in prostate cancer development.* *Update: Current Junior at Huston-Tillotson University*
Nangah Tabukum	*Kexin Xu, Ph.D.*	*Recent studies highlight the roles of bromodomain (BRD) module in crosstalk with AR-signaling network to drive castration resistant prostate cancer (CRPC), and therefore provide proof-of-concept for targeting BRD-containing proteins as therapeutic targets for the treatment of advanced prostate cancer. ATAD2, a bromodomain (BRD)-containing protein, is frequently overexpressed in a broad spectrum of tumors with poor prognoses, including CRPC, and it has been identified as an AR co-activator. Analysis of cancer genomics data sets from prostate cancer patient samples, which Nangah Tabukum was involved, showed that ATAD2 is the most frequently amplified BRD protein in metastatic, hormone-refractory prostate tumors. The main project that Nangah carried out focuses on the proteomic analysis of ATAD2-interacting proteins in order to fully understand its biological function in prostate cancer cells. She was able to construct ATAD2-expressing plasmid with FLAG/HA tandem tags and transfect into LNCaP cells. If with more time, she will continue with tandem affinity purification and mass spectrometry analysis of ATAD2-associated proteins.* *Update: Current Sophomore at Huston-Tillotson University*
Tiarra Walker	*Denise O'Keefe, Ph.D.*	*Methylation profiling of prostate tumors from men that had later either progressed to metastatic disease, or who had no evidence of disease for at least five years, revealed potential methylation biomarkers for metastatic disease. The goal of this project was to identify likely markers that could be used to develop clinical assays to help predict which patients should be more closely watched clinically. Tiarra used a new method, high resolution melt analysis to develop assays for three of the genes she identified as potentially important. Furthermore, as methylation likely regulated these gene transcripts, we tested this in*

		an in vitro model, as future therapies may be able to be designed targeting these changes. *Update: Current Junior at Huston-Tillotson University*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Robin J. Leach, Ph.D. Updates:

Elucidating the Effects of Obesity on Bladder Cancer Progression - completed

CTRC at UTHSCSA: Genomics Shared Resource; reduced from 1.2 cal months to 0.96 cal months

Texas Cancer Diagnostics Pipeline Consortium - completed

The Cancer Bioinformatics Initiative: A UTSA/UTHSCSA Partnership; reduced from 2.16 cal months to 0.6 cal months – no cost extension ending in August 2017.

NCI, R01, Improving prostate biopsy efficiency: The finasteride challenge test - completed

The San Antonio Center of Biomarkers of Risk for prostate cancer (SABOR); renewed, but new effort is 0.12 cal months

Cancer Therapy & Research Center – CURE Supplement; new award
NIH/NCI
$221,814
8/1/16-7/31/19
0 cal months (part of parent grant)
Funded through the Diversity Training Program of the National Cancer Institute aims to increase the number of underrepresented populations engaged in basic, translational and population-based biomedical cancer research by providing a rich training environment for both promising high school and undergraduate students who are currently students in the San Antonio area of South Texas.

Improving the detection of prostate cancer in men with low PSA; new award
UTHSCSA CTRC
$60,000
7/15/16-8/31/17
1.2 cal months
The goal of this current study is to evaluate 30 men with abnormal urinary biomarkers and normal PSA with imaging and offer them prostate biopsies.

MUSC Transdisciplinary Collaborative Center in Precision Medicine & Minority Men's Health, U54MD010706; new award
Medical University of South Carolina/NCI
$ 354,745
7/08/16-3/31/21
0.6 cal months

The overarching goal of the Medical University of South Carolina (MUSC) Transdisciplinary Collaborative Center (TCC) in Precision Medicine for Minority Men's Health is to determine the most effective ways to integrate, interpret, and apply biological, social, psychological, and clinical determinants of disease risks and outcomes into more precise medical strategies to prevent, diagnose, and treat chronic health conditions and diseases.

Detecting prostate cancer in men with low PSA; new award
The William & Ella Owens Medical Research Foundation
$100,000
4/1/2017-3/31/2018
0.24 cal months
To improve the utility of PSA for the early detection of prostate cancer, we propose to develop a new assay that will detect the presence of this variant in serum from men who are being screened for prostate cancer.

The University of Texas Adult Clinical Center (UTACC) U01AR071150; new award
UT Medical Branch at Galveston
University of Texas Adult Clinical Center, NIH Molecular Transducers of Physical Activity Consortium (MoTrPAC)
$39,396
12/07/2016-11/30/2022
0.12 cal months
The purpose of the MoTrPAC is to catalyze the identification and mapping of molecular and cellular transducers underlying the physiological effects of physical activity.

Denise O'Keefe, Ph.D. Updates:

Oncogenic LINE-1 Retroelements Sustain Prostate Tumor Cells and Promote Metastatic Progression - completed

Folate and PSMA interact to regulate DNA methylation in the prostate; reduced from 4.8 cal months to 0.36 cal months, no cost extension – NCI – R01

An Interventional Study to Reduce Folate Levels in Men on Active Surveillance for Prostate Cancer; new award
UTHSCSA CTRC
$39,556
8/15/2016-8/14/2017
0.12 cal months

Novel Regulation and Oncogenic Mechanisms of Fatty-Acid Synthase (FASN) in Aggressive Prostate Cancer, W81XWH-17-1-0244; new award
DOD
$858,752
7/15/2017-7/14/2020
3.6 cal months
Our overall hypothesis is that dietary folate has a novel regulatory role in FASN expression and function through modulation of AMPK action and/or epigenetic modulation that is mediated by PSMA.

Teresa Johnson-Pais, Ph.D. Updates:

Texas Cancer Diagnostics Pipeline Consortium – completed

Prostate SPORE Pilot: Biobanking and Pathology; new award
UTHSCSA CTRC
$46,401
12/15/2016-12/14/2017
0.12 cal months

Recruiting and Retaining Underrepresented Students R25GM095480; new award
NIH
$374,215
5/1/17-4/30/2018
Here we propose a series of systematic interventions designed to sharpen critical thinking skills and develop grant and manuscript writing as well as presentation skills. Successful integration of these strategies will prepare IMSD scholars for the most competitive positions in the biomedical workforce.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *Not applicable*
QUAD CHARTS: *Not applicable*

9. APPENDICES:

Posters of all students included.

Immunohistochemistry (IHC) Assay on Mouse Mammary Tissue

SCHOOL OF MEDICINE
UT HEALTH SCIENCE CENTER
DEPARTMENT OF UROLOGY

Johnson Bobmanuel, Xiang Gu, Hakim Bouamar, Lu-Zhe Sun
The University of Texas Health Science Center at San Antonio

HUSTON-TILLOTSON UNIVERSITY
1875 AUSTIN, TEXAS

Introduction of IHC

- Immunohistochemistry(IHC)=Immunology+histology+chemistry.
- The main purpose of IHC is to use antibodies to detect protein expression on tissue samples.
- In order to achieve this goal, I will be using the IHC assay to detect the protein expression on mammary stem cells / tissues from mouse mammary gland.
- A mammary gland comprises of ducts with working units "made of two layers, an inner layer called the Luminal cells and an outer layer called the [2] myoepithelial cells which is confined to a basement membrane called basal cells."
- It is evident today that scientists have discovered that "the very rare population of stem/progenitor cells within the tumor is present in various tissues [1] and some of this stem cells give rise to tumors." Previous studies have shown that this population of stem cells are resistant to therapy because each time we try to kill a tumor, the tumor shrinks and grows back after a while.
- Recent discoveries in stem cell biology have shown that these "tumors contain self-renewal property [1] that stimulates tumorigenesis" and most often the stem cell initiating tumor are resistant to therapy.

Methods

When doing IHC, there are certain parameters to put into consideration. They include;

•**Antibody selection (primary and secondary) :** When choosing the antibody, one needs to look-up information about the host of the primary antibody from the manufacturer or datasheet which will further determine the choice of secondary antibody. It should not be randomly picked but carefully selected. For instance, the primary antibody is K8 and the host is rabbit, therefore the secondary antibody will be biotin goat anti-rabbit.

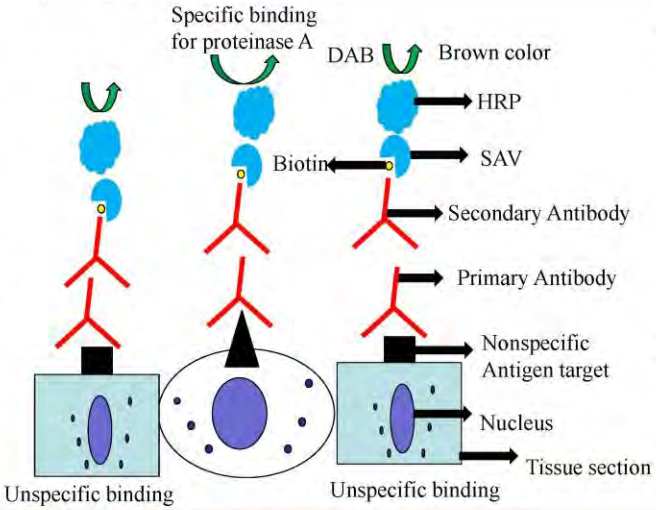
•**Fixation:** Its function is to preserve the shape and localization of the protein. Some epitopes may not be altered over fixation. The tissue used for this experiment was fixed in Carnoy's fixative solution overnight.

•**Sectioning:** Tissue section is recommended to be cut at 3-5um and placed in water bath of about 37 degree Celsius and allowed to dry for about two hours before commencing IHC.

•**Antigen retrieval:** This is a very crucial step which allows the site of the protein to be recognized by the antibody by unfolding. It does this by undoing the inter and intra-protein bridges that have been made by the fixative solution which unmasks and expose the epitope to the antibody.

•**Blocking:** This will help to neutralize the unspecific binding by mimicking the secondary antibody. The primary antibody will recognize the antigen from the goat serum instead of the unspecific proteins from the tissue.

How it works



Results

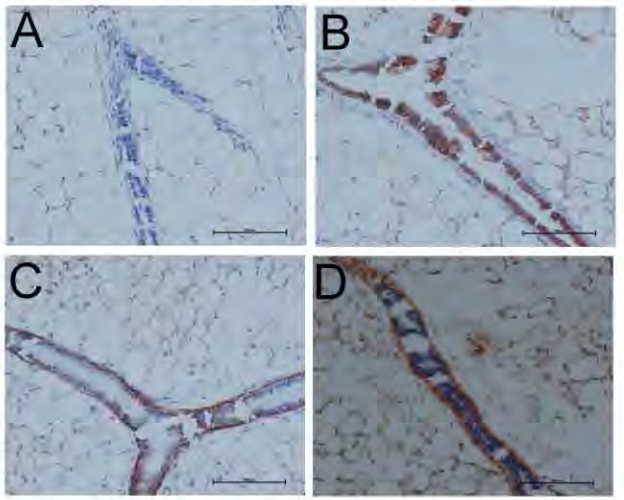


Figure 1. IHC staining results A: Negative control, B: K8 Antibody stains the luminal layer, C: SMA Antibody stains the basal layer, D: K14 Antibody stains the basal layer.

Conclusion

The above result was obtained after many attempts of a 2-day IHC protocol. The proteins were well expressed on the mouse mammary gland but the background stain was relatively high. The IHC protocol is crucial and tricky, it is a powerful tool and can be carried out at a much more lower cost. However, the pitfall for IHC is that we can stain just one protein at a time. To overcome this problem in the future, I would make use of the Immunofluorescence assay which is more sensitive and more than one antibody can be used at a time.

References

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The effect of metformin and folate on prostate cancer growth

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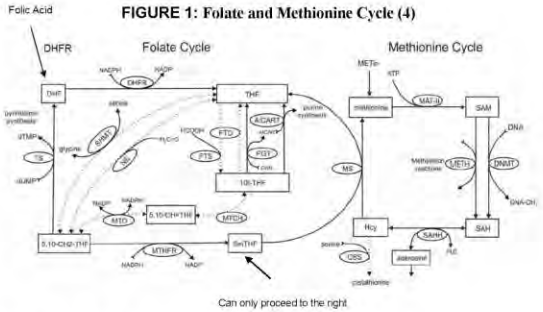
Introduction

Prostate cancer is the most common type of cancer in American men and the second most common cause of cancer death (1). Metformin, is an FDA approved oral drug usually utilized in the treatment of type 2 diabetes to improve blood sugar levels in diabetic patients (2). Recent studies have demonstrated that the use of metformin, results in decreased incidence and improved outcomes for prostate cancer(3). There are ongoing clinical studies examining the effect of metformin in patients with advanced prostate cancer. However the exact mechanisms by which metformin exerts its anti-tumorigenic effects are disputed, with some reports indicating that it may be through its ability to activate AMPK, and inhibition of Mtor (2, 3). However other reports have revealed that its antitumor effects are modulated through regulation of the folate-one carbon pathway (3). In this experiment, we are looking at the effect of metformin and different forms of folate on prostate cancer growth and folate regulation.

Abstract

Metformin was used to treat cells of the LNCaP cell line and its sub-line M. Luc LNCaP, in order to observe the effects on folate metabolism and its regulatory genes. Metformin was added in different amount of concentrations to Folic Acid and 5-Methyltetrahydrofolate (5MTHF) which is the natural form of folate found in the human body. Increased levels of Folic Acid was hypothesized to increase proliferation of LNCaP cells by increasing the conversion of Folic Acid to DHF and then DHF to THF by DHFR. This will result in an increase in the production of nucleotides for cellular replication. 5MTHF was hypothesized to alter the growth of LNCaP cells by shifting folate metabolism toward the methionine cycle, thus increasing methylation of DNA and not producing as many of nucleotides as an equal amount of folic acid.

FIGURE 1: Folate and Methionine Cycle (4)



Materials and Methods

Measurement of LNCaP Cell growth

In order to measure the growth of LNCaP cells, LNCaP cells were cultured in RPMI media. A luciferase assay was performed by plating M.LUC LNCap cells on 24 well plates with various concentration of metformin (0, 2 and 5 mM) in 0 folate, 50nM folic acid, 50 nM 5MTHF, 2.3 uM folic acid and 2.3 uM 5MTHF for a period of 7 days. During the duration of the experiment, triplicate samples from all different treatments were collected each day at exactly 24 hours from when the pervious samples were collected. The amount of luciferin secreted via luciferase was measured in the M. Luc LNCaP cells and graphed based on the time period of growth. The graph was used to measure the growth response of the cells in response to altered folate and metformin.

RNA Extraction and Reverse Transcriptase

Plated cells from all different concentration treatments were then harvested using TRIZOL to extract RNA, which was subsequently quantified and utilized for cDNA synthesis via reverse transcriptase. Real time Polymerase chain reaction(PCR) was done for(FOLH 1, PCFT and RCF which are all folate regulating gene as well as GUSB which was the control since it is a normal house keeping gene) from all cDNA samples that were made form the different treatments.

Results

We initially grew the cells in regular RPMI media, which contains 2.3 uM Folic Acid as its folate source, in the presence of 0, 1, and 5 mM Metformin for 3 days. We then examined the expression of folate hydrolase 1 (FOLH1), proton-coupled folate transporter (PCFT), and the reduced folate carrier (RFC), 3 molecules that are involved in cellular folate uptake.

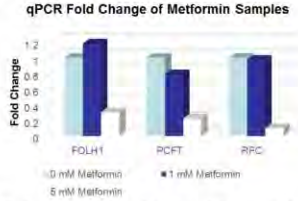


FIGURE 2. Metformin significantly reduced the expression of FOLH1, PCFT, and RFC.

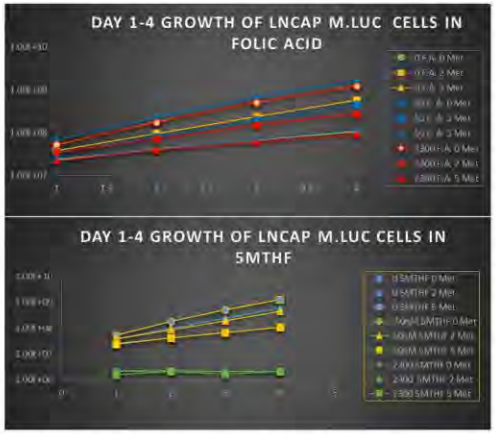


FIGURE 3: Top: A decrease in cell growth was observed with increasing concentration of Metformin, as well as slightly decreased growth with increasing folic acid. Bottom: A similar effect was seen in cells when growing in 5MTHF, however, at 2300 nM the majority of the cells died.

After obtaining results from the first experiment, a second experiment was conducted with just 5MTHF on two different 24 well plates from different companies (BD Falcon and Costar) to determine if plate composition was affecting cell growth. Cells were plated in halving concentrations of 5MTHF starting from 2400 nM ranging to 0 nM without Metformin to solely observe the effects of 5MTHF. The experiment was done for a 6 days.

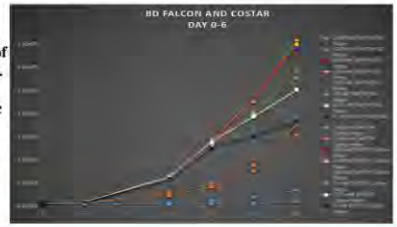


FIGURE 4: 5MTHF treatment of M. Luc LNCaP cells over 6 days. The highest levels of 5MTHF killed a significant portion of the cells at 24 hours, however, the surviving cells then grew at the same rate. After day 4, the cells at the lower 5MTHF levels slowed down in growth.

Cells in the 2.3 uM 5MTHF were unable to be harvested for RNA extraction due to the majority of cells not surviving. The other cells still viable at the other concentrations on the 24 well plates were used to extract RNA and make cDNA for qPCR analysis at 7 days.

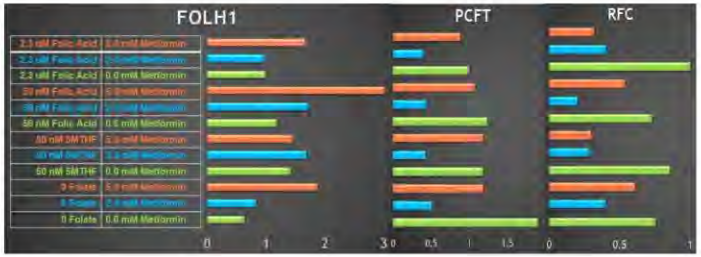


FIGURE 5: The treatment of the cells with different concentrations of metformin and increased folate after 7 days altered the expression of FOLH1, PCFT, and RFC.

Conclusion

Based on the results obtained, it appears that Metformin treatment of LNCaP cells is correlated with a change in expression of genes involved with folate metabolism. Although Metformin treatment alone resulted in a decreased expression of FOLH1, PCFT, and RFC, when Metformin treatment was paired with varying levels of folic acid and 5MTHF an increase in expression of FOLH1 was observed, which could be explained by the different time points of 3 versus 7 days, but also indicating a need for further experiments.
The lack of production of luciferin in the LNCaP cells treated with high levels of 5MTHF indicate that a large proportion of cells died, which was not expected. Interestingly after the initial 48 hours of cell death, the cells that had survived in the higher concentrations began to grow at the same rate as the cells that were at lower concentrations of 5MTHF. Further experiments are necessary to determine that exact biochemical mechanisms occurring.

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RPS6KB1 inhibition sensitizes prostate cancer cells to chemotherapy?

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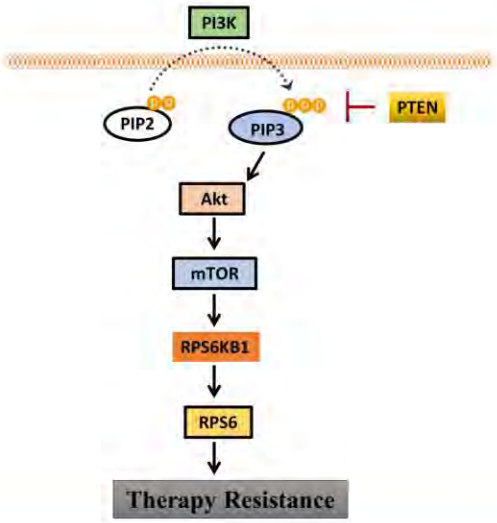
Introduction

- Prostate cancer (PCA) is the second leading cause of cancer related death in U.S men.[1]
- Castration resistant prostate cancer (CRPC) has a mean survival of only 19 months.
- Docetaxel (Dox) & Enzalutamide (ENZ) are currently used for CRPC treatment.[2]
- However, Dox and ENZ cause a minimal increase in survival (~5 months) and patients develop resistance.[2-3]
- Hence, there is need to find novel CRPC treatments.
- PI3K/mTOR pathway is deregulated in 40% localized PCA patients and almost all CRPC patients.[4]
- RPS6KB1, is a downstream effector of mTOR pathway, which controls protein translation, cell proliferation, cell survival.[5]
- Role of RPS6KB1 in PCA is not well studied.
- We tested if downregulation of RPS6KB1 sensitizes PCA cells to Dox and ENZ.



Results

RPS6KB1 inhibition decrease anchorage-dependent and independent growth

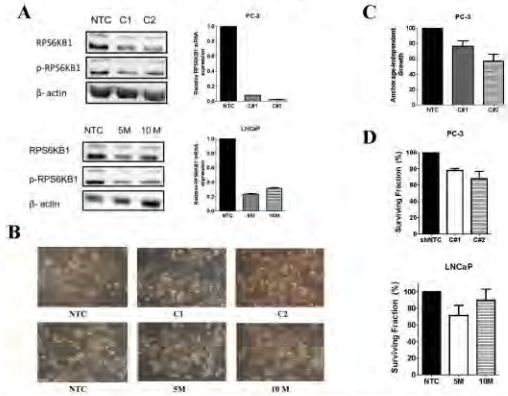


Figure 1: (A) Validation of RPS6KB1 knockdown at protein and RNA level in PC-3 (NTC, C1, C2) and LNCaP (NTC, 5M, 10M) PCA cells. (B) Phase-contrast images of PC-3 and LNCaP cell lines, stably silenced for RPS6KB1. (C) Anchorage-independent growth was measured in PC-3 (NTC, C1, C2) cells using soft-agar assay. (D) Surviving fraction of PC-3 (NTC, C1, C2) and LNCaP (NTC, 5M, 10M) cells using colony formation assay.

RPS6KB1 inhibition sensitizes PCA cells to Dox

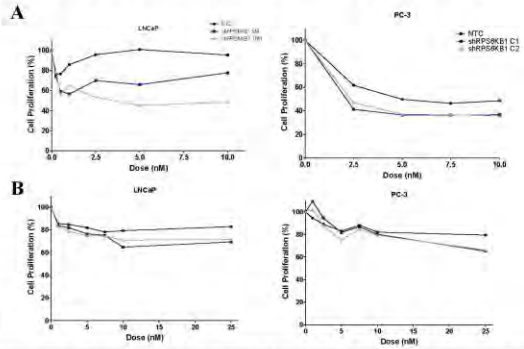


Figure 2: (A) LNCaP (NTC, 5M, 10M) and PC-3 (NTC, C1, C2) were treated with different doses of Dox for 72h and MTT assay was performed to measure cell viability. (B) LNCaP (NTC, 5M, 10M) and PC-3 (NTC, C1, C2) were treated with different doses of ENZ for 72h and MTT assay was performed to measure cell viability.

RPS6KB1 levels are higher in human prostate tumors

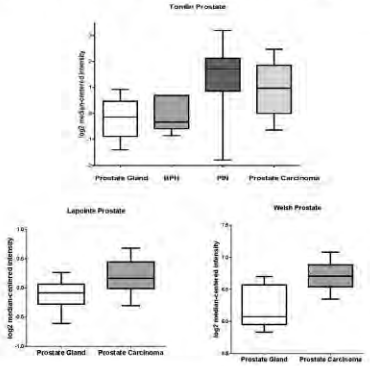


Figure 3: RPS6KB1 expression in normal prostate, benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN) and prostate carcinoma, analyzed from Oncomine clinical databases using Graphpad Prism V.

Conclusions

- RPS6KB1 does not affect cell morphology, but decreases both anchorage independent and dependent cell growth.
- RPS6KB1 inhibition sensitizes PC-3 and LNCaP cells to Dox treatment.
- RPS6KB1 inhibition does not affect ENZ sensitivity in PC-3 & LNCaP cells.
- PCA patients have higher expression of RPS6KB1.

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Acknowledgements

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Cognitive Impairment Associated with Androgen Deprivation Therapy

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Introduction

- 47- 65% of prostate cancer patients who undergo Androgen Deprivation Therapy (ADT) exhibit significant cognitive impairment in areas such as memory, visuo-spatial ability, attention and executive function.
- The medial prefrontal cortex (mPFC) is important to many of these cognitive processes. fMRI studies indicate hypoactivity in the mPFC, reduced gray matter volume, and reduced functional connectivity of the mPFC at rest in ADT patients compared to those who did not undergo this treatment.
- Also, clinical studies show that ADT produces the most prominent impairment in visuospaital cognition, such as spatial recognition and memory. These domains are mediated by the hippocampus (Hpc).
- Vortioxetine, a novel mulitmodal antidepressant with characteristics similar to an SSRI and has a specific efficacy for the cognitive impairment seen in depression.

Methods

Animals and Drug Dosage:

- Sprague Dawley male rats (intact & physically castrated) weighed 225-249g upon arrival.
- Rats were singly housed and received either the control or vortioxetine diet.
- Vortioxetine diet (0.6 g/kg corresponding to a dose of approx. 28 mg/kg/day) was administered for 17 days prior to testing. This included ten days of free-feeding followed by seven days of food restriction (14 g/day).

AST (Attentional Set-Shifting Test):

- Rats must learn to associate a cheerio reward with positive stimuli: texture of the digging medium or the odor on the rim of the pot.
- 6 consecutive correct trials are required before moving on to the next phase.
- In the extra-dimensional set-shifting task, the positive stimulus becomes negative, requiring a shift in cognitive set, which is dependent on the mPFC.

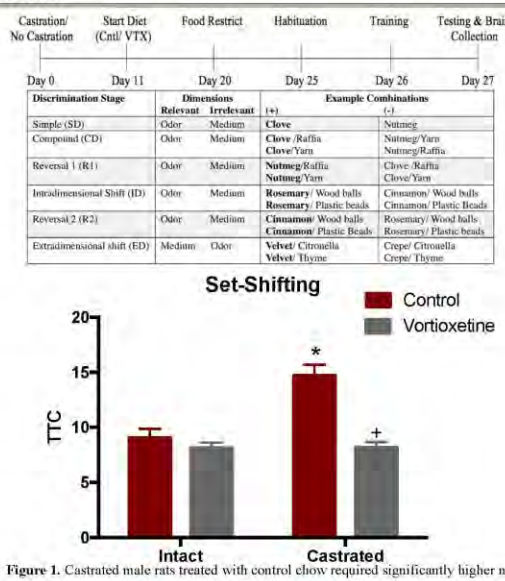
NOL (Novel Object Recognition Test):

- Evaluates rodent's ability to recognize that one of two familiar objects in the environment has changed location.
- Evaluate the interaction time with the object in the novel location versus the total interaction time with both objects.
- Discrimination Ratio (DR) = (Tn-Tf)/(Tn+Tf).
- This task requires visuospatial recognition and memory, which are mediated by the Hpc.

Evoked Field Potential Recordings:

- Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and body temperature was maintained at 37° C for duration of procedure.
- A stainless steel stimulating electrode was placed in the right medial dorsal thalamus (from bregma, DV: -5.4, AP: -2.6, ML: +0.9 mm) while a second stainless steel recording electrode was placed in the right mPFC (DV: -3.5, AP: +3.0, ML: +0.6).
- Local field potentials were recorded in the mPFC after stimulation in the MDT.
- Data represented as a current-response curve by stimulating the MDT with 30 pulses (100-600 µA in 100 µA increments, 260-µs pulse width, 0.1 Hz).

Results



Discrimination Stage	Dimensions Relevant	Dimensions Irrelevant	Example Combinations (+)	Example Combinations (-)
Simple (SD)	Odor	Medium	**Clove**	Nutmeg
Compound (CD)	Odor	Medium	**Clove** /Raffia **Clove**/Yarn	Nutmeg/Yarn Nutmeg/Raffia
Reversal 1 (R1)	Odor	Medium	**Nutmeg**/Raffia **Nutmeg**/Yarn	Clove /Raffia Clove/Yarn
Intradimensional Shift (ID)	Odor	Medium	**Rosemary**/ Wood balls **Rosemary**/ Plastic beads	Cinnamon/ Wood balls Cinnamon/ Plastic Beads
Reversal 2 (R2)	Odor	Medium	**Cinnamon**/ Wood balls **Cinnamon**/ Plastic Beads	Rosemary/ Wood balls Rosemary/ Plastic beads
Extradimensional shift (ED)	Medium	Odor	**Velvet**/ Citronella **Velvet**/ Thyme	Crepe/ Citronella Crepe/ Thyme

Figure 1. Castrated male rats treated with control chow required significantly higher number of trials to criterion on the set-shifting task than intact male rats treated with control chow (*p<0.0001). Chronic vortioxetine treatment reversed the deficit in set-shifting performance in castrated male rats (+p<0.0001). All data are presented as mean ± SEM, n=10-12/ group.

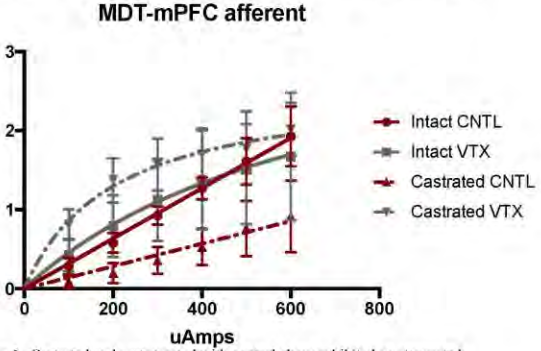
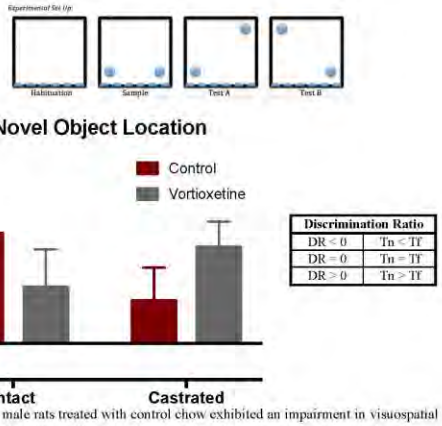


Figure 2. Castrated male rats treated with control chow exhibited an attenuated electrical response in the mPFC evoked by stimulating the excitatory afferent input from the MDT, compared to intact rats treated with control diet. Chronic dietary vortioxetine normalized the evoked response of castrated male rats to a level comparable to that seen in intact control rats. All data are presented as mean ± SEM, n=4-6 per group.



Discrimination Ratio	
DR < 0	Tn < Tf
DR = 0	Tn = Tf
DR > 0	Tn > Tf

Figure 3. Castrated male rats treated with control chow exhibited an impairment in visuospatial memory as indicated by a lower discrimination ratio than than intact male rats treated with control chow. Chronic dietary vortioxetine treatment reversed the deficit in castrated male rats. All data are presented as mean ± SEM, n=4-7/group.

Conclusion

- Androgen deprivation by physical castration in rats produced a cognitive deficit in both AST and NOL, which models the human cognitive impairment seen after ADT.
- Also, vortioxetine treatment reversed the deficit in cognitive performance in castrated male rats, suggesting vortioxetine may be effective in treating cognitive impairment in ADT patients.
- Finally, androgen deprivation caused a decrease in evoked afferent response in the mPFC, while chronic vortioxetine treatment increased evoked response in the mPFC after stimulating in the MDT.

Future Directions

- Investigate changes in functional plasticity by stimulating excitatory afferents from the ventral Hpc and recording evoked field potentials in the mPFC.
- Use Golgi stain technique to assess the structural changes in the mPFC.
- Characterize the differences in gene expression, focusing on signaling pathways, transmitters, and modulators that are regulated by androgen that are important to cognition.
- Clinical trials are on-going to test the effects of vortioxetine on cognitive impairment in prostate cancer patients who undergo ADT.

Acknowledgements

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Common Supplements can augment the efficacy of Valproic Acid in treating Prostate Cancer

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Abstract

Rising prostate specific antigen (PSA) levels in prostate cancer (CaP) patients treated with surgery or radiation is known as biochemical recurrence (BCR). BCR indicates disease relapse, which often is treated with androgen deprivation therapy (ADT). ADT has multiple morbidities which may compromise quality of life and there is controversy regarding early or late use of ADT for BCR. There is lack of a clear overall survival advantage with the use of immediate ADT, and alternative treatment options that could eliminate or delay ADT would be beneficial. Castrate-resistant prostate cancer (CRPC) is the lethal **phenotype** and therefore the target of current therapy. There have been numerous studies demonstrating the utility of histone deacetylase inhibitors (HDACIs) in treating CRPC. We have studied Valporic acid, an HDACI, in treating CaP as well as bladder cancer in animal models. Docosahexaenoic acid (DHA) an omega-3 Fatty Acid (FA), is a primary structural component of the human brain, skin, sperm, testicles and retina. Fish oil is a great source of DHA, and is a popular nutritional supplement. Even though there is controversy concerning the benefits of DHA, there are over two decades of research that show beneficial effects of DHA on bone health, as well as a chemo-sensitizing agent in treating cancer. Similar efficacy of cell kill by VPA can be achieved with half the dose when combined with DHA. This potentially can reduce the side effects associated with VPA treatment. Chronic treatment with DHA, VPA, as well as the combination results in epigenetic changed in the treated cells. Folate, which provides the methyl group in DNA methylation, also enhanced cell kill when combined with DHA & VPA.

Results

Variable	Observation	VPA	p-value
Number of participants	6	6	
RP Age (years)	62.7 ± 9.5	63.2 ± 8.1	0.92
Mean ± Std Dev (Range)	(46.0-72.0)	(51.0-72.0)	
Gleason	8.0 ± 1.4	7.2 ± 0.8	0.23
Mean ± Std Dev (Range)	(6.0-10.0)	(6.0-8.0)	
PSA at RP	10.6 ± 7.9	15.5 ± 8.8	0.47
Mean ± Std Dev (Range)	(5.1-19.5)	(7.8-26.0)	
PSA at the Time of Enrollment	4.2 ± 2.5	3.4 ± 2.9	0.60
Mean ± Std Dev (Range)	(2.3–9.0)	(1.5-9.2)	
PSADT (Months) Prior to Enrollment	6.3 ± 2.1	4.0 ± 2.8	0.84
Mean ± Std Dev (Range)	(4.4-8.9)	(0.8-8.8)	
PSADT (Months) at end of Study	7.0 ± 3.8	40.7 ± 46.1	0.03
Mean ± Std Dev (Range)	(3.7-12.5)	(3.5-100.0)	

Figure 1: Demographics of the participants in a Randomized, Controlled Phase II Study of Valproic Acid in Patients with Non-Metastatic Biochemical Progression of CaP

Acknowledgements

U.S. Army Medical Research Acquisition Activity Grant W81XWH-16-1-0217
Drs. R. Rodriguez, D. Bacich, D. O'Keefe, and W. H. Chowdhury

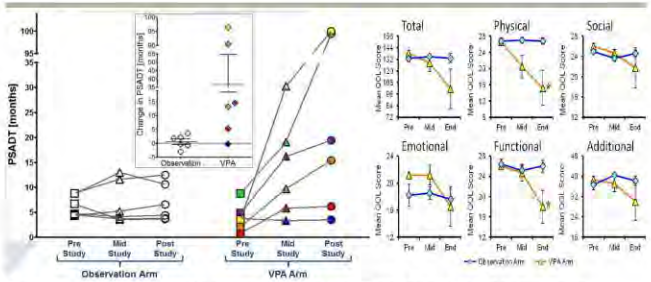

Figure 2: VPA treatment resulted in the increase in PSA Doubling time in Prostate cancer patients with biochemical recurrence. The treatment also resulted in a loss in quality of life.

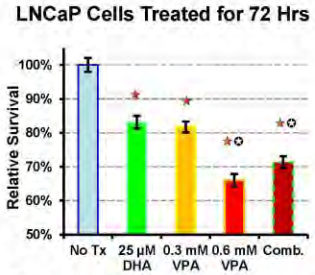

Figure 3: LNCaP cells were plated in 96 well plates and treated with DHA, VPA, and a combination (25 uM DHA + 0.3 mM VPA) for 72 hours. The addition of 25 uM DHA was able to reduce the requirement of VPA by almost half the dose.

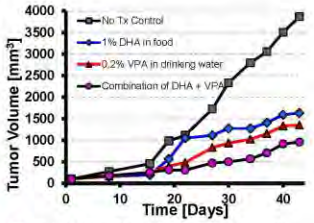

Figure 4: Nu/Nu mice with sub-cue LNCaP xenograft were fed defined diet containing DHA or Sun flower oil, and provided with 0.2% VPA in drinking water. The rate of tumor growth was reduced to 20.3 mm^3/day in the combination group as compared to the VPA (34.0 mm^3/day), DHA (41.8 mm^3/day), and no treatment (97.5 mm^3/day)

Conclusion

- Combining DHA with VPA can reduce the dose of VPA required for cell kill.
- The combination of a common dietary supplement such as DHA has the potential to reduce the side effects of VPA , as a reduced dose could be used to treat patients.
- DHA, VPA, and the combination treatment resulted in changes in methylation in the control elements of genes involved in tumorigenesis, tumor progression, differentiation, metabolism, signal transduction.
- The addition of Folic Acid, another common dietary supplement further enhances the cell kill by VPA.
- Patients need to be advised on effects of dietary supplements, as they can positively (as seen here) or even potentially negatively effect the efficacy of certain drugs.

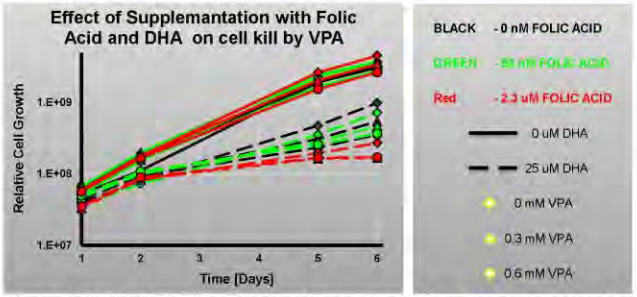

Figure 5: LNCaP-M.LUC cells were plated in three 24 well plates, in increasing concentrations of folic acid (0 nM, 50 nM, 2.3 uM), and treated with DHA, VPA and a combination (25 uM DHA + 0.3 mM VPA). The combination, in the presence of high folic acid had the largest cell kill compared to the cells treated with the other drug conditions.

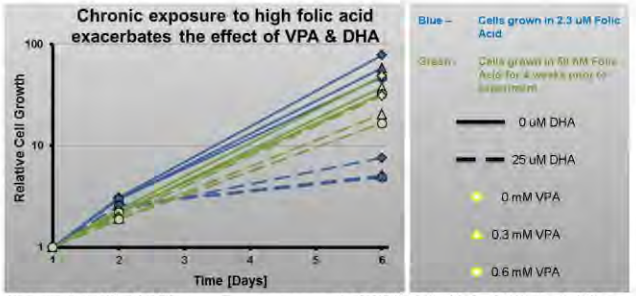

Figure 6: LNCaP-M.Luc cells were grown in RPMI1640 (2.3 uM Folic Acid) or in RPMI containing 50 nM Folic Acid for 4 weeks before repeating the experiment described in figure 5. The effect of high folic acid on the increased cell kill is diminished when the cells are grown in physiological levels of folic acid before conducting the experiment.

Changes in Methylation upon chronic treatment with DHA, VPA or a combination:

LNCaP cells were grown in RPMI-1640 with 10% FBS containing 10 nM DHA, 0.3 mM VPA, or a combination of the two drugs for 110 days. The genomic DNA was isolated from these cells and changes in methylation were analyzed using the Infinium MethylationEPIC Kit.

Some of the genes that are differentially methylated in both the DHA & VPA treatment but further enhanced in the combination are: MAPK10, PDGFD, FAM110B, FMN2, GPM6A, UNC80, ADCY8, LTBP1.

Some of the genes that are Differentially methylated at a much higher rate in the combination treatment are: MAPK10, STAT5A, FHIT, PDE1A, CFTR, LIMA1, STAT3, RABGGTB, MIR21, FPR3.

Does AMPK activates AR in human prostate cell line?

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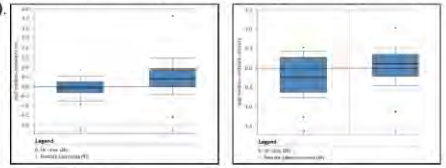
Introduction

- Prostrate cancer is the second most leading cause of cancer related deaths amongst American men.
- AMPK is a stress sensor that is expressed in all the cells. It consists of three subunits (alpha-catalytic, beta and gamma- regulatory subunits).



- AMPK is activated in PCA due to hypoxia and metabolic stress found in tumor cells. Activation of AMPK restores cellular energy balance by promoting ATP generating processes which helps in cell survival.
- The role of AMPK in context dependent and its role in PCA is not clear.
- Objective is to test whether AMPK promotes or inhibits PCA cell growth.

Results

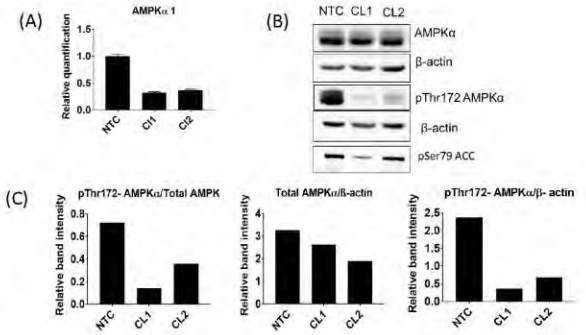


FIG 1: Validation of AMPKα1 Knock-down in LNCaP cell Lines.
A)Total RNA from Logarithmically growing NTC and two stable knock-down clones of AMPKα1 in LNCaP cells was used for qPCR. Analysis of the qPCR was done using Bio-Rad CFX manger. B) and C) Cell extracts from NTC, Cl1 and Cl 2 were prepared in SDS loading buffer. Immunoblot analysis was performed with total AMPKα, phosphorylated AMPKα(Thr172) and phosphorylated ACC (Ser79ACC). β-actin was used as loading control. Immunoblots were developed using GENESYS Software and quantification was done using GENETOOL Software. Both clones show significant decrease levels of pThr172 and pSer79ACC.

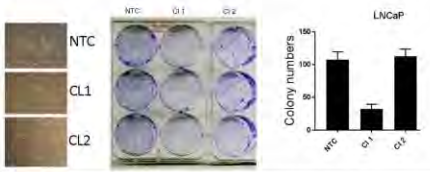


FIG 2. Suppression of AMPK inhibits growth in LNCaP cells?
A) 100,000 cells were seeded with complete media in a 12 well plate and after 48 hours pictures were taken at 20x magnification. Clones do not show much difference in morphology.
B)5000 cells were seeded in a 6-well plate. Seeding was done in triplicate and allowed to form colonies for 12-14 days. Colonies were stained with 0.05 % crystal violet in methanol and surviving cell fraction was calculated manually. Colonies having less than 500 cells were not counted. Silencing AMPK stably reduces colony forming ability of LNCaP cells in clone 1 (CL1) with no effect on clone 2 (CL2)

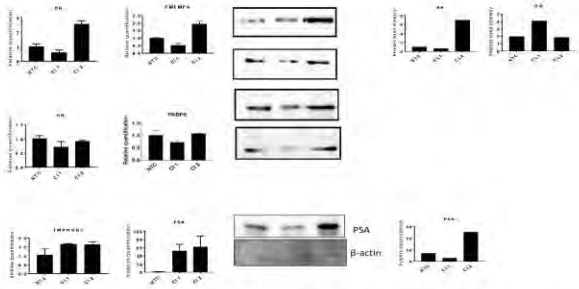


FIG 4. AMPK silencing reduces AR activation?.
mRNA expression and protein levels of AR,GR and target genes were determined by qPCR(A) with AR,GR,PMEPA1,FKBP5,TMPRSS2, PSA specific primers and western blot (B) with AR,GR and PSA-specific antibody. AMPK Knock down decreases AR activation in CL1 but increases in CL2 as evidenced on both mRNA and protein levels. All target genes (PMEMPA1 and PSA) also show activation in same clones.

Conclusion

- AMPKα1 knockdown was confirmed in LNCaP cells at mRNA and Protein level.
- Similar level of knock- down shows differential biological outcome. In clonogenic assay CL1 shows fewer number of colonies with no significant effect on CL2.
- Stress causing agents including androgen deprivation may help knock-down cells to proliferate more.
- AMPK knock-down makes cells more sensitive to AR antagonist, Enzalutamide and chemotherapeutic agent Docetaxel.
- Sensitivity to the drugs depends on the amount of AMPK present in the cells

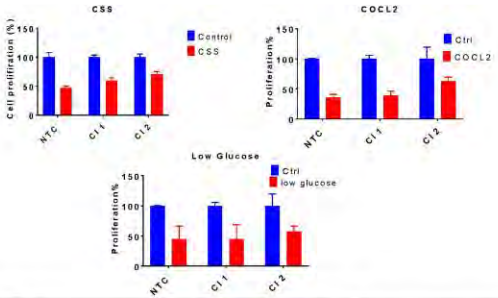


FIG 3. The stress response of AMPKα1 knock-down on LNCaP cells.
NTC and knock-down clones were treated with stress causing agents as CoCl2 (hypoxia), CSS (androgen deprivation) and Low Glucose (nutrient deprivation). MTT assay was performed after 72 hours to determine cell proliferation. Result shows that AMPKα1 is essential for cell survival under above stress.

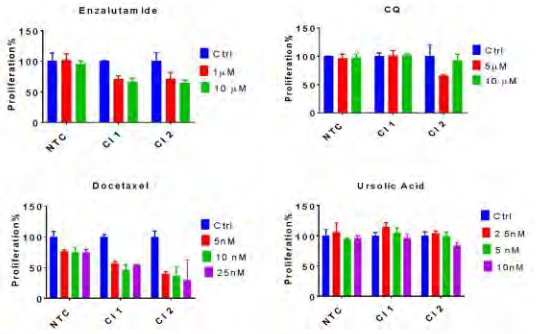


FIG 5. Different effects of drugs on AMPKα1 knock-down cells
4000 cells were seeded in 96 well plate after 48 hours respective drugs were added. 72 hours after addition of the drugs, MTT assay was performed to analyze cell proliferation. Silencing AMPK appreciates the sensitivity of LNCaP cells to treat increased AR antagonist (Enzalutamide) and cytoskeletal targeting agent (Docetaxel). Inhibition of autophagy or NFkB activation via Chloroquine and Ursolic acid had no effect.

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Acknowledgements

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Plasmid Construction- A way to study the role of ATAD2

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Introduction

- Androgen receptor (AR) plays a pivotal role in prostate cancer, primarily by regulating different gene expression programs elicited by androgen, which is important for cancer cell proliferation, survival, and differentiation.
- ATAD2, a new member of the AAA+ ATPase family proteins, as a novel AR coactivator. ATAD2 interacts directly with AR and enhances its transcriptional activity, and is required for androgen-stimulated expression of a specific subgroup of genes.
- Although ATAD2 is hardly detected in normal human prostate tissue, high levels of ATAD2 are found in hormone-independent prostate cancer cell lines, xenograft tumor, and a subset of prostate cancers with high Gleason scores. These findings suggest that ATAD2 plays an important role in prostate cancer by mediating specific AR functions in cancer cell survival and proliferation (1).

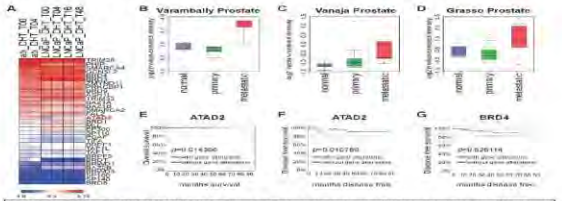


Figure 2. Upregulation of ATAD2 in advanced prostate cancer with poor prognoses. (A) Heat-map of expression levels of human bromodomain family members in LNCaP and abl cells. Cells were treated with vehicle (T00) or DHT for various time periods (1hr, T04; 16hr, T16; and 48hr, T48). (B-G) Three independent microarray datasets showing elevated *ATAD2* expression in hormone-refractory, metastatic prostate cancer (red), compared to the normal prostate (blue) and primary cancer (green) with statistical significance. (b-G) Kaplan Meier plots of genetic alterations in *ATAD2* (E and F) and *BRD4* (G) from the TCGA prostate tumor cohorts.

Kexin Xu, unpublished.

Purpose

To construct a plasmid containing Flag-HA and ATAD2 in order to find out and further analyze the role it plays in cell regards to cell survival and proliferation in prostate cancer.

Flowchart

Construct Plasmid ➡ Generate Virus ATAD2-Flag- Ha ➡ Infect target cells ➡ coimmunoprecipitate HA-tag and Flag—tag

Materials and Methods

Backbone- Flag- HA
Insert- ATAD2
Primers- ATAD2-F,ATAD2-R
Protocols

- •Ligation Protocol with T4 DNA Ligase (M0202)
- •PCR Using Q5 Hot Start High-Fidelity DNA Polymerase(M0493)
- •QIAquick PCR Purification Kit using a Microcentrifuge
- •Plasmid DNA Purification Using the QIAprep Spin Miniprep Kit and a Microcentrifuge
- •Midi-Prep (Boyer Lab)
- •Gene Subcloning Procedure

Steps

1. **Choose the vector and analyze its restriction sites**
2. **Preparation of interest DNA to be cloned**
   - Amplify template DNA using PCR (M0493)
   - Target size – 4kb; confirm thru gel
3. **Purification of the PCR products**
   - Follow protocol QIAquick PCR Purification Kit Using a Microcentrifuge (4kb target size)
   - Confirm size, check for non specific bands and concentration(185ng/ul)

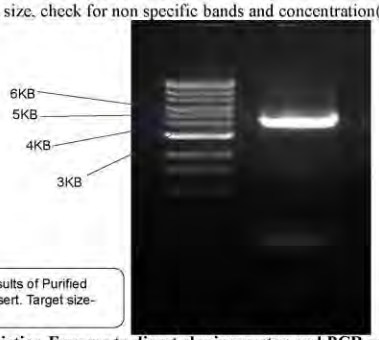


Fig 2. Results of Purified ATAD2 insert. Target size- 4KB.

4. **Use Restriction Enzyme to digest cloning vector and PCR products**
   - Tube #1(vector) - 5ul + 1.5ul NotI + 5ul buffer 3.1+ 38.5 H2O
   - Tube #2 (ATAD2) – 5.4ul +1.5ul NotI + 5ul buffer 3.1 +38.1 H20
   - Leave in 37°c overnight
5. **Dephosphorylating Vector**
   - To reduce chance of self ligation
   - Add 3ul of Rsap to Vector
   - Store in 37°c for 30 minutes
   - Then purify using "QIAquick PCR Purification Kit Using Microcentrifuge" INSERT PIC
   - Concentration- vector -4.7ng/ul (8kb)
   - Insert-13.2 ng/ul (4kb)
6. **Ligation with T4 DNA ligase (M0202)**

Vector	12.5ul	12.5ul	12.5ul
ATAD2	8ul	13ul	17ul
T4 DNA ligase buffer	2ul	2.8ul	3ul
Ligase	1ul	1ul	1ul
water	-	-	-

7. **Transformation**

Add 100ul DH5X – 20 to 30 mins on ice – 42°c for 45 seconds – 2 mins on ice – Add 800 ul LB (Amp -) – Shake in 37c for an hour- 3000rpm for an hour – discard supernatant - leave 150ul on Amp+ LB plate – Incubate in 37°c overnight.

- Select clones form each ration and dilute with 10ul of water
- PCR 5ul to check for correct direction of clone insertion

Fig 3. PCR results checking for positive clones from transformation

5ul + 700ul of Amp+ LB - shake all day - once positive clones are confirmed with remaining 5ul thru PCR and gel – Add 7ml AMP+ LB to clones – shake overnight in 37°c

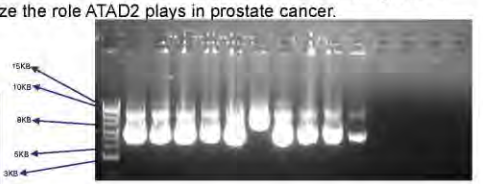
- Purify positive clones (PCR) and measure concentrations
- Run gel to confirm band size and check for successful ligation (12kb)
- Send ligated samples for DNA sequencing
- Repeat Transformation step on positives clones only

8. Follow Midi Prep Boyer Lab Protocol to extract Plasmids

Conclusion

Through gene analysis on cells derived form prostate cancer patients, we successfully confirmed the upregulation of ATAD2 in metastatic prostate cancer. We successfully constructed a plasmid containing ATAD2. The next logical step would be to use the constructed plasmids to infect target cells and further analyze the role ATAD2 plays in prostate cancer.

Fig 4.sample #10-vector(8kb).Successful ligation of ATAD2 insert(4kb) and vector(8kb) can be seen on sample #6 (12KB)



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Acknowledgements

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Regulation of LIN7A by Methylation in Prostate Cancer Cell Lines

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Introduction

Prostate Cancer (Pca) is the second most common cancer in American men. It is now estimated that there will be approximately 161,360 new cases of PCa and about 26,730 deaths from PCa this year alone. Around one in seven men develop PCa during their lifetime. PCa is more common in older men, with an average age of 65 at time of diagnosis. In efforts to reduce PCa mortality, Prostate Specific Antigen (PSA) screenings have been utilized to detect prostate cancer prior to symptomatic disease. However, this has caused a concern of over treatment in low grade cancers. Biomarkers have become an essential tool in PCa by helping to create a more personalized approach for patients, and identifying whether the disease will become more aggressive over time. A novel area of research for PCa biomarker is DNA hypermethylation. DNA methylation (a process of adding a methyl group to cytosines within the genome) is an epigenetic alteration which can impact gene expression. Regions that are differentially methylated in tumors with different outcomes can be useful as potential biomarkers for aggressive prostate cancer. This study aims to identify regions that are differentially methylated between PCa patients with good outcome and those with poor outcome. Additionally, we plan to confirm the methylation-dependent regulation of genes associated with these DNA regions in PCa cell lines.

Abstract

Previous data was generated from a genome-wide methylation array comparing primary prostatectomy on a group of patients with a minimum of 5 year clinical outcome data. They were distinguished either as No Evidence of Disease recurrence (NED) or Metastasis (MET). Using this data we identified 3 gene regions that were hyper-methylated in METS, (CDCA7L, LIN7A, PDZD4). These genes were tested to see if they are regulated by methylation using PCa cell lines treated with decitabine (methylation inhibitor) and measuring gene expression through qPCR. PC3 and LNCaP prostate cancer cell lines were treated with decitabine and DNA and RNA were then isolated from those cells to be converted into bisulfite DNA for High Resolution Melting (HRM) and cDNA for Reverse transcription. The LNCaP proved to have little to no change between the vehicle and treated for all three genes. There was a slight change in the genes CDCA7L and PDZD4 for PC3. LIN7A demonstrated a four fold increase after treatment, suggesting that LIN7A is regulated by methylation in these cells. HRM analysis showed no evidence of methylation in the CDCA7L region but the primers for LIN7A were inconclusive. Using Mexpress, it was found that methylation of this LIN7A region correlated significantly with expression in prostate tumors in the Texas Cancer Genome Atlas data (TCGA).

Materials and Methods

Decitabine Treatment: 7x10^5 PC3 or LNCaP cells were harvested in T25s and treated with .5ng of decitabine for 7 days. Media and drug were refresh daily. RNA and DNA were isolated for gene expression and methylation analysis.
Gene Expression: 1ug of isolated RNA was converted to cDNA using Applied Biosystems Reverse Transcription Kit. Gene expression was then performed using primers for CDCA7L, LIN7A, and PDZD4. GUSB was used as reference gene. qPCR was performed in a LightCycler 96 (Roche).
Bisulfite Conversion: Bisulfite conversion was used on the DNA isolated using the EZ DNA methylation kit to obtain bisulfite converted DNA (B.S. DNA)
High Resolution Melting (HRM): B.S DNA was used to test methylation with B.S Specific or methylation specific primers. Melt curves for vehicle and treated DNA were identified and compared to controls of 0% methylated DNA, 100% methylated, and a 1:1 combination of 0 and 100%. Methylated DNA is expected to having a higher melt peak due to the higher CG content of the product after bisulfite conversion

Results

Figure 1. Primers Designed against regions from previous data from methylation array.

Gene	Feature	P.Value	NED_AVG	MET_AVG	DeltaBeta
CDCA7L	TSS200	0.009773	0.079909	0.33078	0.250871
CDCA7L	TSS200	0.002359	0.093724	0.311995	0.218271
CDCA7L	TSS200	0.002765	0.079751	0.296154	0.216403
CDCA7L	TSS200	0.026024	0.062715	0.247426	0.184711
LIN7A	TSS200	0.010551	0.077126	0.262141	0.185014
LIN7A	1stExon	0.035111	0.095302	0.279251	0.183949
PDZD4	Body	0.016578	0.091571	0.305426	0.213855

Figure 2. Gene expression after .5nM decitabine treatment in PC3 and LNCaP.

Figure 3. No Significant change in methylation of CDCA7L region as shown by High Resolution Melt with Bisulfite converted DNA.

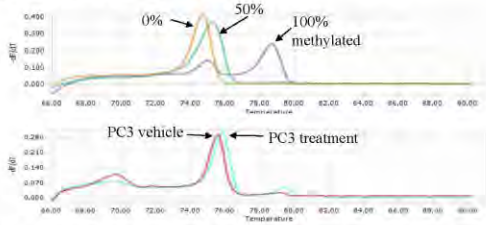
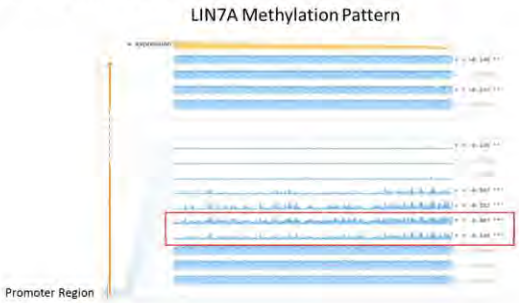


Figure 4. Mexpress Data from the Texas Cancer Genome Atlas.



Conclusion

1. We found three gene regions hyper-methylated in the primary tumor of patients that later progressed to metastatic disease.
2. LIN7A showed a significant change in gene expression between PC3 vehicle and PC3 decitabine treatment.
3. LIN7A methylation correlates with expression in prostate tumors according to TCGA.
4. LIN7A methylation represents a potential biomarker as well as a potential pathway of intervention for treatment.
5. CDCA7L and PDZD4 also represent biomarkers but do not appear to be regulated by methylation in Pca cell lines.

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